Factors controlling biogeochemical removal of nitrogen in constructed wetlands

by Zulimar Lucena

Abstract

The Long Island Sound (LIS) estuary is affected by summer hypoxia as a result of high nitrogen loads from New York and Connecticut watersheds. In order to mitigate hypoxia, managers have established a goal of reducing the nitrogen load from nonpoint sources by 10%. One strategy to reduce N loads from nonpoint sources is the use of constructed wetlands, which provide an ecosystem service by removing pollutants from stormwater runoff. This study examined the effectiveness of constructed wetlands in Hamden and Woodbridge, Connecticut in improving the quality of stormwater runoff. Our main objective was to determine the factors that contribute to N removal to provide design recommendations that optimize constructed wetlands performance. A total of 9 to 21 storms were monitored at four sites during the summer and fall of 2013. Weirs and water level loggers were installed at the inlet and outlet of the wetlands to measure water flow. Stormwater samples were collected using ISCO autosamplers at regular intervals over the duration of storm events. These were composited to obtain flow-weighted samples from the inlet and outlet of each wetland to determine nitrogen loads and mean concentrations per storm event. We also surveyed each site to determine plant diversity, sediment organic carbon concentration, and treatment ratios to determine their influence on N removal. Only two sites showed statistically significant biogeochemical removal of N. Our results indicate that wetland heterogeneity and interspersion between open water and vegetation, as well as high sediment carbon concentrations, promote N concentration reduction. Additionally, we examined the effects of input N concentrations, storm size and intensity, and water temperature using multiple linear regression. The models showed that only influent N concentration influences N concentration reduction. Based on our results we recommend designing interspersed wetlands that offer more opportunities for a variety of biogeochemical processes to occur and using sediments with high carbon concentrations to promote denitrification. Considering these variables might result in more effective N concentration reduction. This information contributes to the limited knowledge of constructed wetland design in Connecticut and can promote higher nitrogen removal rates from stormwater in the Long Island Sound watershed.

INTRODUCTION

Summer hypoxia in the Long Island Sound estuary is driven by excess nitrogen loading from inland sources and has been of major concern for decades. Its harmful ecological consequences – fish kills, changes in food-web structure, and alterations to the life-cycles of aquatic biota (Howell and Simpson 1994, Breitburg et al. 2009, Zhang et al. 2010) – have forced state and federal agencies to implement measures to reduce nitrogen (N) loading and improve the water quality of the Long Island Sound (LIS). In 2000, a Nitrogen Total Maximum Daily Load (TMDL) analysis (NYDEC and CDEP 2000) established a target to reduce N loads in the LIS by 58.5% within 15 years. To attain this goal, upgrades to sewage treatment plants, which contributed approximately 70% of the N loads to the LIS (NYDEC and CDEP 2000), have been a priority management strategy in New York and Connecticut. However, non-point source pollution, including stormwater runoff, also needs to be continuously addressed to successfully reduce N loads. In particular, as urban land use expands and new stormwater sources are added to the existing infrastructure, stormwater management will become increasingly important to achieve established goals.

Connecticut plays a critical role in improving the health of the LIS ecosystem. It is the second largest contributor of N to the LIS and the only state with its entire coastline facing the estuary. All of Connecticut lies within the Long Island Sound watershed, covering approximately 35% of the total watershed area of which 7.5% is impervious surface land cover (Hurd et al. 2006). For nonpoint sources the TMDL assessment established a 10% reduction of N, thus a number of measures to achieve this target have been implemented, including modifications to the stormwater permit requirements and sewer separation projects. Nevertheless, further reduction of stormwater N loads will require the implementation of structural management practices and alternative source control methods.

Constructed wetlands, which provide ecosystem services by removing water pollutants, are an alternative to incorporate into current N control strategies that could yield greater load reductions. These systems were originally employed to treat wastewater from industrial processes and domestic sewage (Kadlec and Wallace 2009), but are also used currently in the treatment of nonpoint source pollution from stormwater runoff . However, a disadvantage of constructed wetlands is that N removal can vary widely. Previous studies have documented both inefficient wetlands that export N as well as systems that remove up to 90% of influent N (Strecker et al. 1992, Vymazal 2007, Lee et al. 2009, Malaviya and Singh 2012). This variability potentially results from particular site characteristics such as unique physical and biogeochemical conditions that influence the processes responsible for N removal (Vymazal 2007, Kadlec 2008, Lee et al. 2009).

N REMOVAL MECHANISMS

Constructed wetlands typically remove N from water in three distinct ways: through hydrological processes, chemical transformations, and assimilation (Bachand and Horne 2000, Vymazal 2007). The main hydrological process leading to N removal from a constructed wetland is *infiltration*.

Influent stormwater infiltrates through the soil, leading to a reduction of N loads at the effluent. Even though N is removed from the wetland, the amount of N transformed or removed in the subsurface is unknown unless measured through observation wells. It is possible that infiltrated N could reach receiving waters through groundwater flow.

The chemical transformations responsible for N removal in wetlands are *ammonia volatilization*, which occurs when pH values exceed 8.0 (Reddy and Patrick 1984b), and *denitrification*, a bacterial process that occurs under anoxic conditions in which nitrate is converted into nitrogen gas (N_2). Denitrification has been identified as the principal mechanism for N removal in wetlands (Gersberg et al. 1983, Reddy and Patrick 1984a, Bachand and Horne 2000, Kadlec and Wallace 2009). However, its effectiveness is dependent on factors such as N concentration, temperature, organic matter quantity and quality, pH, soil type and microbial flora (Vymazal 1995).

Another mechanism for N removal is *assimilation*. In this process, microorganisms, algae, and plants draw inorganic N from the sediment and water column and convert it into organic N, a useable nutrient that can be readily incorporated into their tissues for growth. In temperate climates, N uptake by macrophytes is a seasonal occurrence that takes place during the growing season of spring and early summer. The rate of nutrient uptake is limited by plant growth rates and nutrient concentrations in plant tissue (Vymazal 2007), which can result in variable N removal depending on the species present in the wetland. Plants that may potentially enhance N removal in constructed wetlands are those with high growth rates, high tissue nutrient content, and high potential for biomass accumulation. In addition, a fraction of N in plant tissue may become unavailable for additional cycling after plant death through peat formation processes and burial.

Currently, there is limited understanding of how the factors associated to N transformation and assimilation influence wetland performance. Therefore, understanding their role is essential for optimizing wetland design and increasing the effectiveness of these systems. These quantifiable factors include: (1) sediment organic matter content, (2) vegetation, (3) treatment ratio, (4) influent N concentration, and (5) water temperature.

Organic matter concentration

Organic matter in wetland sediment provides carbon and energy to heterotrophic denitrifying bacteria, potentially influencing N removal rates. Spieles and Mitsch (2000) found that nitrate removal through denitrification may be carbon limited in young wetlands due to the insufficiency of organic matter accumulation. Because denitrification is considered a significant driver of N removal in wetlands, carbon availability may be a limiting factor that can influence removal rates.

Vegetation

The effects of wetland vegetation on N removal are both direct and indirect. Plants contribute directly to the reduction of N in the water column and sediments through assimilation. However, plant uptake has been found to remove only a small fraction of all nitrate in the water column (Bachand and Horne

2000). It has been suggested that vegetation mainly contributes to N removal through its supply of carbon that denitrifying bacteria use as an energy source (Kadlec and Wallace 2009). The quality and lability of this carbon depends on the plant species and determines the rate at which the carbon will be used (Bachand and Horne 2000, Hernandez and Mitsch 2007). In constructed wetlands, *Typha sp., Phragmites sp.,* and *Scirpus sp.* are the most commonly planted species. They have been found to be effective for N removal, but studies recommend a mixture of emergent and submergent vegetation to increase denitrification (Weisner et al. 1994, Bachand and Horne 2000, Liang et al. 2011). Vegetation also affects denitrification rates through the rhizosphere. Plant root tissue provides a suitable environment for microbial attachment and increases the surface area available for this purpose (Kadlec and Wallace 2009).

Treatment ratio

The ratio between the wetland area and that of the contributing watershed is a sizing guideline occasionally used to design stormwater wetlands as it can be related to water residence time. It has been suggested that area ratios of 2% or more result in higher wetland performance (Strecker et al. 1992). However, a study of 49 wetland systems showed that a 2% area ratio results in only a 10% removal efficiency of Total Nitrogen (TN), and that percentage increases exponentially as areal ratios increase (Carleton et al. 2001).

Influent N concentration

Hammer and Knight (1994) observed that TN removal efficiency increases with influent N concentration, which was attributed to the N-fixation potential of the wetland. At low input concentration, internal production and release of N can exceed assimilation, resulting in negative removal efficiencies.

Water temperature

In temperate regions, seasonal variations in temperature may have an effect on nitrogen release and removal. For example, a portion of organic nitrogen is always returned to the water column during the breakdown of detritus and soil organic matter, however, the N release associated with this process is highest during the summer months (Kadlec and Reddy 2001). Seasonal variations have also been related to denitrification rates. Several studies have observed higher N removal rates in warmer months rather than winter months (Stober et al. 1997, Bachand and Horne 2000, Spieles and Mitsch 2000, Kadlec and Reddy 2001) due to higher metabolic rates of denitrifying bacteria when temperatures increase. However, some studies have shown contradicting results as not all systems exhibit this change in N removal with temperature variations (Phipps and Crumpton 1994, Sirivedhin and Gray 2006).

Obtaining a clear understanding of how these factors influence N removal in constructed wetlands is essential to improving the design and efficiency of these systems. Moreover, given the current state of Long Island Sound and the N reduction loads in Connecticut, increasing our knowledge of constructed wetlands functions could lead to more efficient stormwater management practices. To achieve this, there is a need to study constructed wetlands in the watershed as climate, physical characteristics, and stormwater composition differ from other regions in which most studies have been conducted.

The purpose of our research was to: (1) evaluate the effectiveness of constructed wetlands in removing N from stormwater through biogeochemical processes in the LIS watershed, (2) identify the key contributing factors of stormwater N removal, and (3) provide wetland design recommendations that lead to maximum N removal for the improvement of the LIS ecosystem. To do this we evaluated biogeochemical N removal, by calculating N concentration reduction, in four constructed wetlands in Connecticut and assessed the factors that influence biogeochemical N removal mechanisms. We expected to observe variable N removal between sites as a result of dissimilar wetland design. In particular, we predicted higher N concentration reduction in wetlands with high organic matter content in sediment, vegetation cover, and treatment ratios. We also hypothesized that N removal would be the highest in warmer months and during storms with high influent N concentrations.

METHODS

STUDY SITES

The study was conducted in four constructed wetlands in south central Connecticut. Two sites, Davis and Thornton, are located in the town of Hamden (Figure 1). These were constructed by the South Central Regional Water Authority to protect the water quality of Lake Whitney, a drinking water supply. The remaining sites, Lois, and Marion, are located in the town of Woodbridge, and were installed to manage stormwater runoff from land development in the watershed. The sites selected vary in size, design, and drainage basin areas (Table 1). To facilitate sampling and hydrology calculations we selected wetlands that were accessible and had only one influent and effluent pipe.

Table 1. Site characteristics

Site Name	**Location**	**Number of ponds**	**Pond Area (m^2)**	**Watershed Area (ha)**	**Treatment ratio (%)**
Thornton	Hamden	2	238	10.9	0.22
Davis	Hamden	3	365	9.7	0.38
Lois	Woodbridge	1	1101	8.5	1.30
Marion	Woodbridge	1	456	2.1	2.17

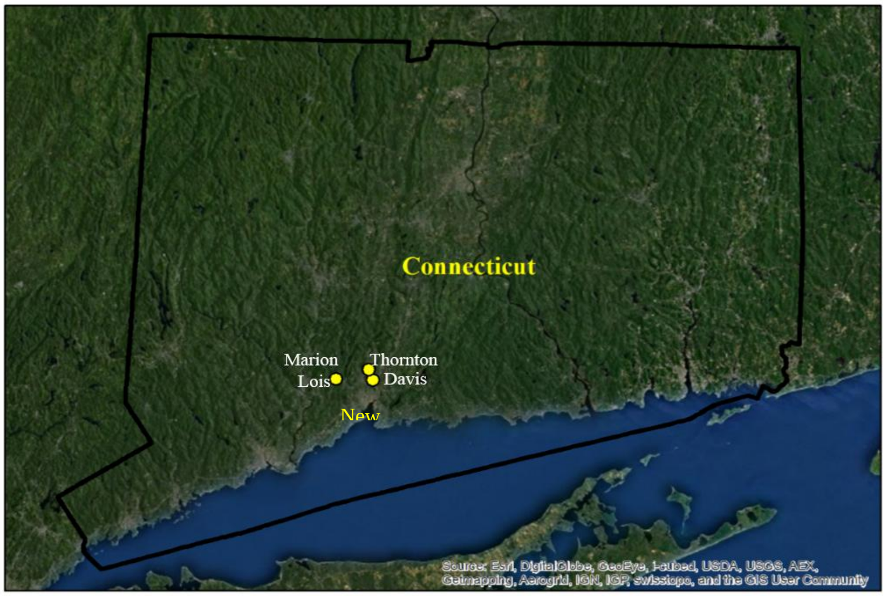

FIGURE 1. MAP OF CONNECTICUT SHOWING STUDY SITE LOCATIONS.

STUDY DESIGN

Our data collection period spanned from April 2013 to December 2013. Due to equipment limitations, we were only able to collect samples at three sites concurrently, resulting in data from different time periods between sites. Additionally, in 2013, the total rainfall amount in the region was 813 mm, lower than the annual average of 1200 mm, and extended periods of little or no rainfall were experienced. This caused a disparity in the amount of storms collected between sites. In total, 21 storms events were collected at Thornton, 12 at Davis, 9 at Marion, and 11 at Lois (Figure 2 to Figure 4). For two storms at Thornton and Lois and one storm at Davis there was inflow, but no outflow.

The range of storm sizes collected was also dissimilar between sites. Samples collected at Thornton include a wider range of storm sizes compared to Davis, Lois, and Marion (Table 2). This is partially due to the fact that only Davis and Thornton were sampled during the month of June, the wettest month of the year. As a result of equipment malfunction we were unable to collect storms at Davis during a period in June. The rest of the year most storm events did not exceed 15 mm (0.7 in) of rain, resulting in samples from smaller storms at the remaining sites.

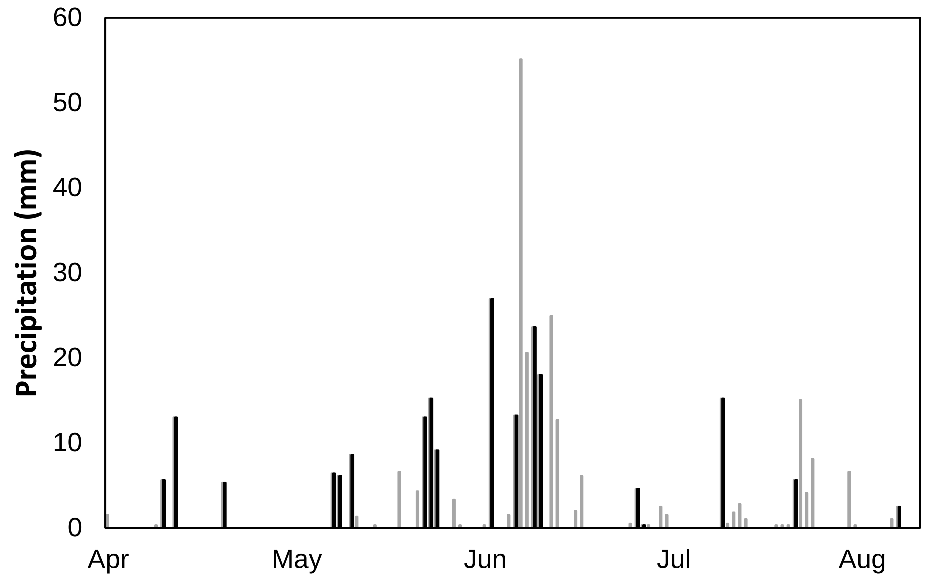

FIGURE 2. SAMPLE COLLECTION PERIOD AT THORNTON. BLACK BARS INDICATE STORMS COLLECTED (N=21). PRECIPITATION DATA FROM TWEED NEW HAVEN AIRPORT WEATHER STATION (SOURCE: NOAA NATIONAL CLIMATIC DATA CENTER).

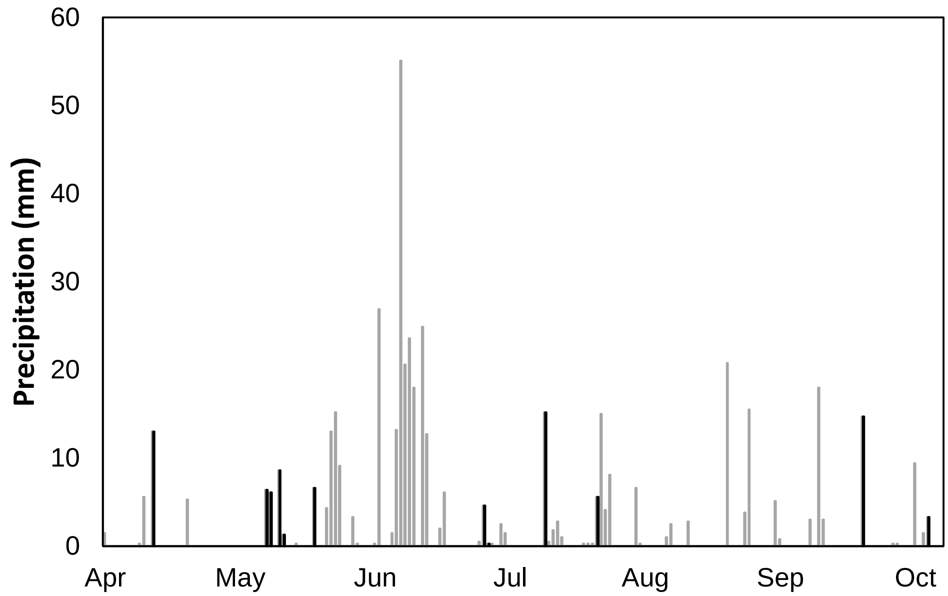

FIGURE 3. SAMPLE COLLECTION PERIOD AT DAVIS. BLACK BARS INDICATE STORMS COLLECTED (N=12). PRECIPITATION DATA FROM TWEED NEW HAVEN AIRPORT WEATHER STATION (SOURCE: NOAA NATIONAL CLIMATIC DATA CENTER).

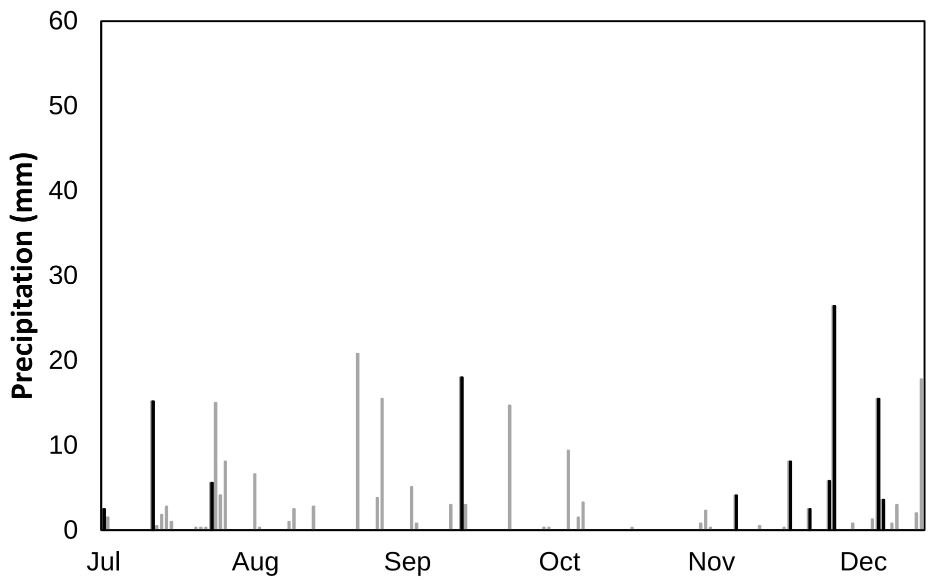

FIGURE 4. SAMPLE COLLECTION PERIOD AT LOIS. BLACK BARS INDICATE STORMS COLLECTED (N=11). PRECIPITATION DATA FROM TWEED NEW HAVEN AIRPORT WEATHER STATION (SOURCE: NOAA NATIONAL CLIMATIC DATA CENTER).

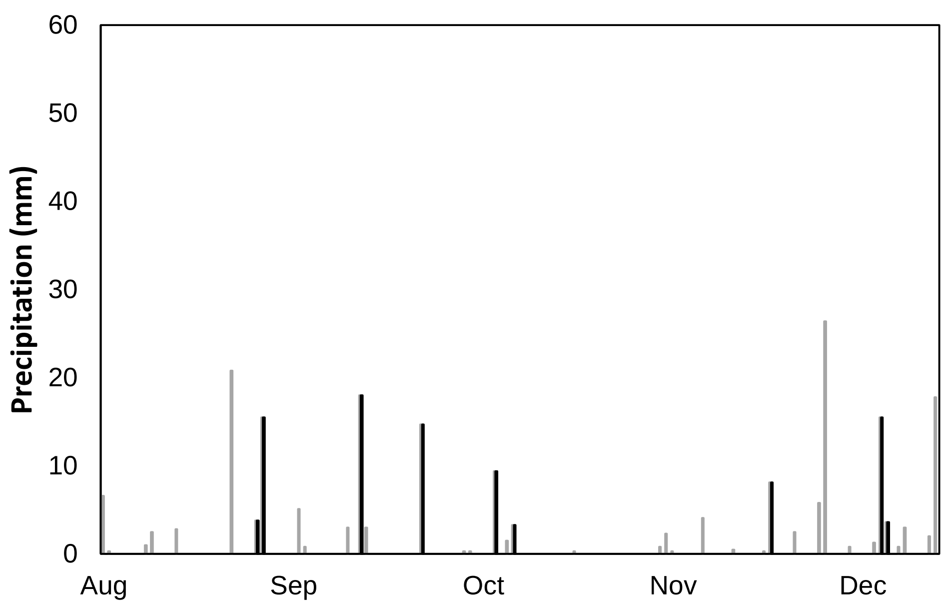

FIGURE 5. SAMPLE COLLECTION PERIOD AT MARION. BLACK BARS INDICATE STORMS COLLECTED (N=9). PRECIPITATION DATA FROM TWEED NEW HAVEN AIRPORT WEATHER STATION (SOURCE: NOAA NATIONAL CLIMATIC DATA CENTER).

Table 2. **Storm characteristic summary**.

Site	Sampling period	Number of storms sampled	Storms with no outflow	Storm size range (mm)
Thornton	April-August	21	2	0.3 - 26.9
Davis	April-October	12	1	0.3 - 15.2
Marion	August-November	9	0	3.3 – 18.0
Lois	July-December	11	2	2.5 - 26.4

To determine nitrogen fluxes, we measured flows and nitrogen concentrations (TN and NO_3^-) at the inlet and outlet of each site. Influent and effluent flows were calculated using stage-based equations and weirs. Because influent and effluent pipe features were variable between sites, appropriate stage-based methods were selected based on site characteristics (Table 3). In most sites, we were able to install weirs and use their associated stage-discharge equations. However, we also applied empirical equations such as the orifice equation and Manning's formula at sites where it was not possible or necessary to install a weir. We measured the water level at the inlet and outlet of each site every five minutes using pressure transducers. Solinst® Leveloggers were placed in the influent and effluent pipes at Davis, Thornton, Marion, and in the effluent at Lois. A Level TROLL® 500 was used in the influent pipe at Lois.

TABLE 3. FLOW MEASUREMENT METHODS USED AT EACH STUDY SITE

Site	Influent	Effluent
Thornton	Cipoletti Weir	Cipoletti Weir
Davis	90° V-notch weir	90° V-notch weir
Lois	90° V-notch weir	Orifice equation
Marion	Manning Formula	Orifice equation

Stormwater samples were collected at the inlet and outlet of each wetland using ISCO 3700 automated samplers. The automated samplers were connected to a liquid level actuator that started sample collection when it detected an increase in water level. Each sample bottle contained five subsamples collected every 6 to 12 minutes during a storm, which was representative of 30 to 60 minutes of flow. In order to capture the first flush of pollutants, when there may be more variation in concentrations, samples were collected every 6 minutes during the first two hours of a storm and later scattered to longer intervals of 12 minutes. Ice was placed in the automated samplers to preserve the samples until collection within 24 hours of the start of the storm.

In the laboratory, the bottles obtained were manually composited into flow-weighted samples within 24 hours of collection. Each composite sample was filtered using 0.45 µm Millipore Durapore filters and stored frozen until sample analysis. Raw and filtered samples were analyzed for total nitrogen (TN), NO_3^-, and Cl^- (ion chromatography). For chemical analyses, quality control measures included replicates, spikes, calibration standards, and quality control standards of known concentration.

Storm and site variables

To determine the factors that influence N removal, we assessed 8 variables. Four variables - treatment ratio, plant diversity, and sediment carbon and nitrogen concentration - are factors that differ between sites but not between storms at a given site. The other variables - influent flow volume (used as a proxy for storm size), maximum flow rate (used as a proxy for storm intensity), temperature, and influent N concentration - vary at each site on a storm by storm basis.

Vegetation was surveyed at all sites between July and August 2013. Because the size of the wetlands was relatively small (≤1,100 m^2) the entire pond area was surveyed. We categorized plant communities within and each wetland and identified all species in each community. The perimeter of the plant communities was marked using a GPS unit to determine their coverage area with ArcGIS. By combining the species data with the community polygons we determined the percent cover of vegetation and species diversity at each site.

To determine sediment organic matter content, samples were collected in August and September 2013. A total of 10 soil samples were collected at each site, each sample a composite of three subsamples of 2 cm of depth taken 1 m apart from each other. To identify sampling points, we used plant community data obtained from the vegetation survey. Once plant communities were identified, random points were generated at each community polygon to obtain the sample location. At each site, we collected an equal number of samples per community. However, the number of samples per community varied by site since the number of communities at each wetland varied from 1 to 5. After collection, samples were homogenized and dried at 70°C. They were also manually ground and analyzed for carbon (C) and N content (FLASH Elemental Analyzer). We calculated average sediment C and N concentrations at each site based on the area of each community from which the samples were collected. We used the following equation:

$$Area\ weighted\ Concentration = \sum \left[\frac{Area\ of\ plant\ community}{Wetland\ area} x\ Mean\ concentration\ in\ community\right]$$

Data Analysis

We calculated event mean concentrations (EMCs) at the influent and effluent of each site for each storm using flow and concentration data from stormwater samples. An event mean concentration is the flow proportional average concentration of a given parameter during a storm event calculated by dividing the total constituent mass discharge by the total runoff volume.

To test if there was significant biogeochemical removal of N, two-tailed one-sample t-tests comparing the mean of concentration change (Δ Conc. = Influent EMC – Effluent EMC) to zero were performed. If the data did not follow a normal distribution, a Wilcoxon signed-ranked test was performed instead of a student's t-test. This was also done for the other water quality parameter measured, but our main focus was on TN and nitrate.

To determine which storm variables (influent N EMC, storm size, and mean water temperature) contribute to N removal, we created multiple linear regression models to predict the concentration change of TN and nitrate. This analysis was performed only with data from the sites that showed significant concentration reduction of N. However, because of our limited number of sites, performing statistical tests to determine the effect of site variables on N removal was not possible. As an alternative, after determining significant biogeochemical removal of N at each site, we did a qualitative analysis of site variables to hypothesize potential factors that could be influencing N removal.

RESULTS

The concentrations of TN, NO_3^-, and Cl^- for samples collected at the inlet and outlet of each site are summarized in

Table 4. Stormwater composition was variable between sites. Davis and Thornton showed a wider range of influent concentrations for TN while the widest range of influent concentrations NO_3^- was observed at Lois.

TABLE 4. SUMMARY OF CONCENTRATIONS OBSERVED AT ALL CONSTRUCTED WETLANDS.

		Thornton		***Davis***		***Lois***		***Marion***	
		Influent	*Effluent*	*Influent*	*Effluent*	*Influent*	*Effluent*	*Influent*	*Effluent*
TN (mg/L)	Median	1.67	1.16	2.96	2.67	2.84	2.45	2.04	1.76
	Min	0.40	0.29	1.65	1.63	1.43	1.17	0.51	0.80
	Max	10.08	5.58	10.93	3.42	5.46	3.40	6.26	5.62
NO3	Median	0.34	0.22	0.64	1.24	1.44	0.98	0.37	0.36
	Min	0.04	0.02	0.17	0.57	0.11	0.68	0.17	0.09
	Max	1.32	0.65	1.63	1.58	4.83	2.19	0.66	0.53
Cl (mg/L)	Median	2.31	2.60	13.52	21.71	5.22	11.00	9.99	9.19
	Min	0.52	0.52	3.55	2.19	1.40	3.92	3.95	4.38
	Max	47.39	56.23	61.38	32.61	33.23	33.44	18.68	19.88

Graphs showing Effluent versus Influent EMCs for all parameters measured are shown in Figures 6 to 8. Results from one-sample t-tests and Wilcoxon tests (for data that did not show a normal distribution) showed a statistically significant reduction of TN only at Thornton ($p<0.05$) and Davis ($p<0.05$). NO_3^- concentration reduction was only significant at Thornton ($p<0.05$). Results were not statistically significant for Cl^- at any of the sites. Because both Cl^- is a conservative tracer, no difference between influent and effluent concentrations was expected.

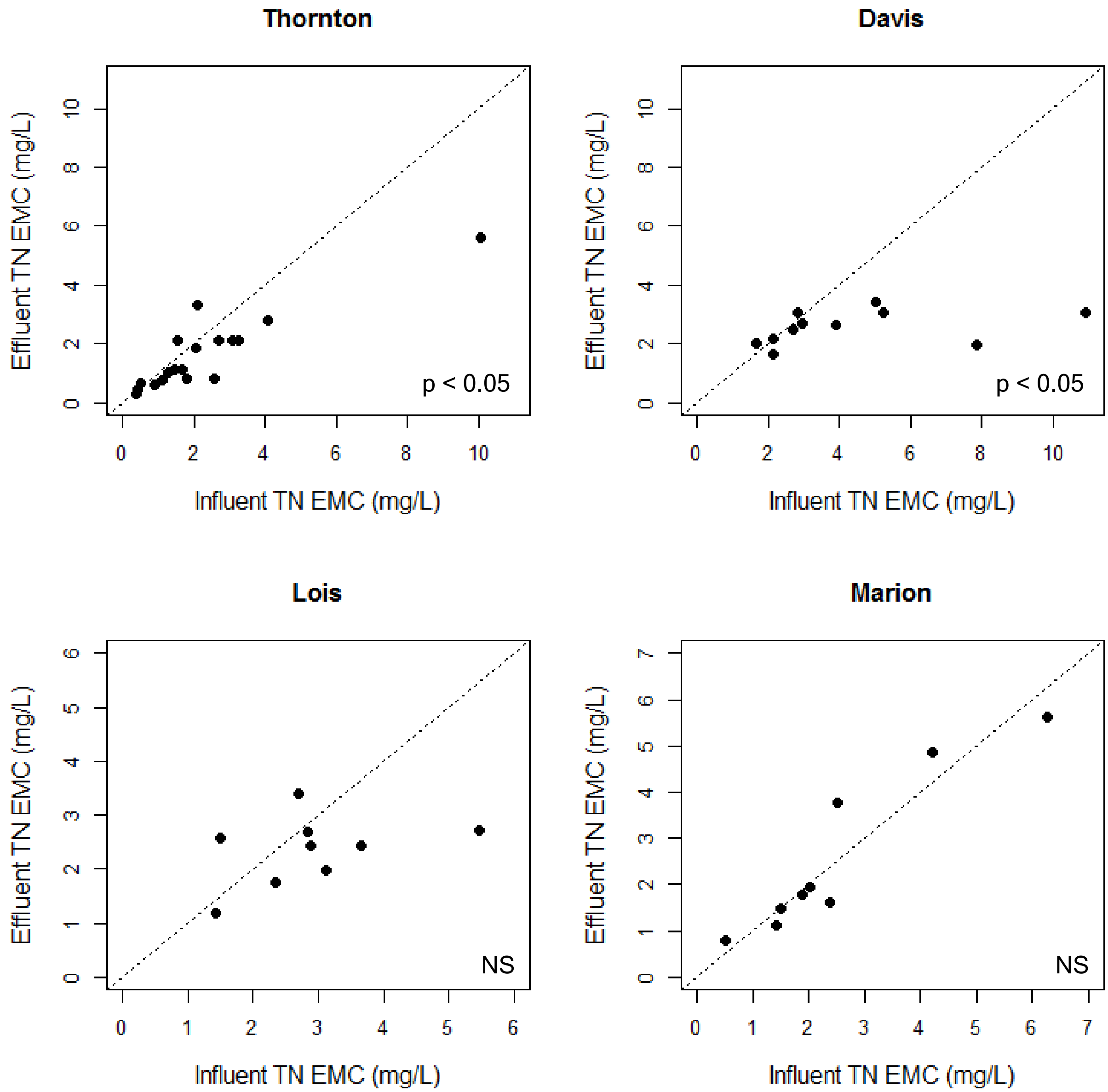

FIGURE 6. EFFLUENT TN EMC VS. INFLUENT TN EMC PLOTS FOR EACH WETLAND. DOTTED LINE REPRESENTS Y = X.

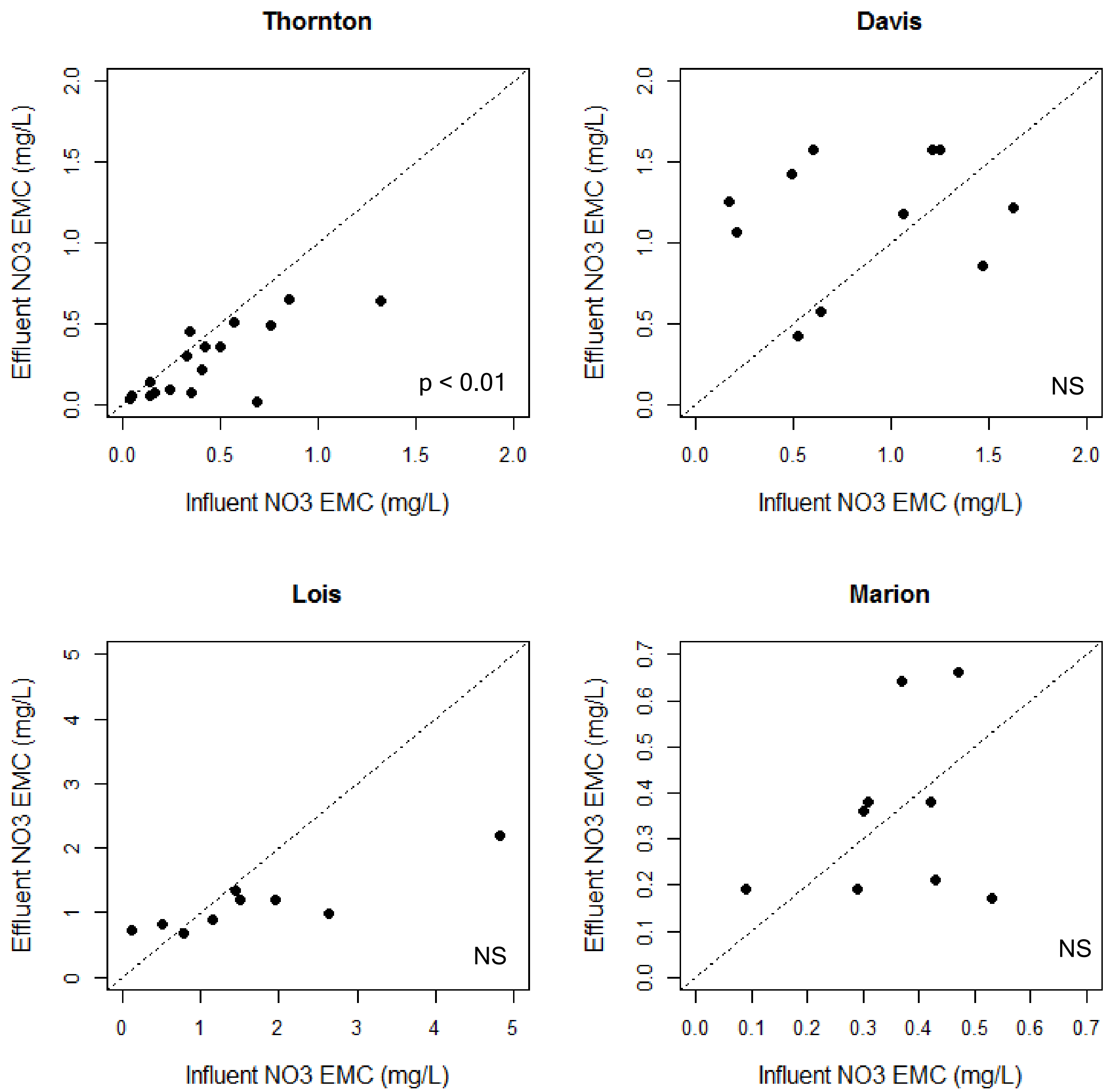

FIGURE 7. EFFLUENT NO3 EMC VS. INFLUENT NO3 EMC PLOTS FOR EACH WETLAND. DOTTED LINE REPRESENTS Y = X

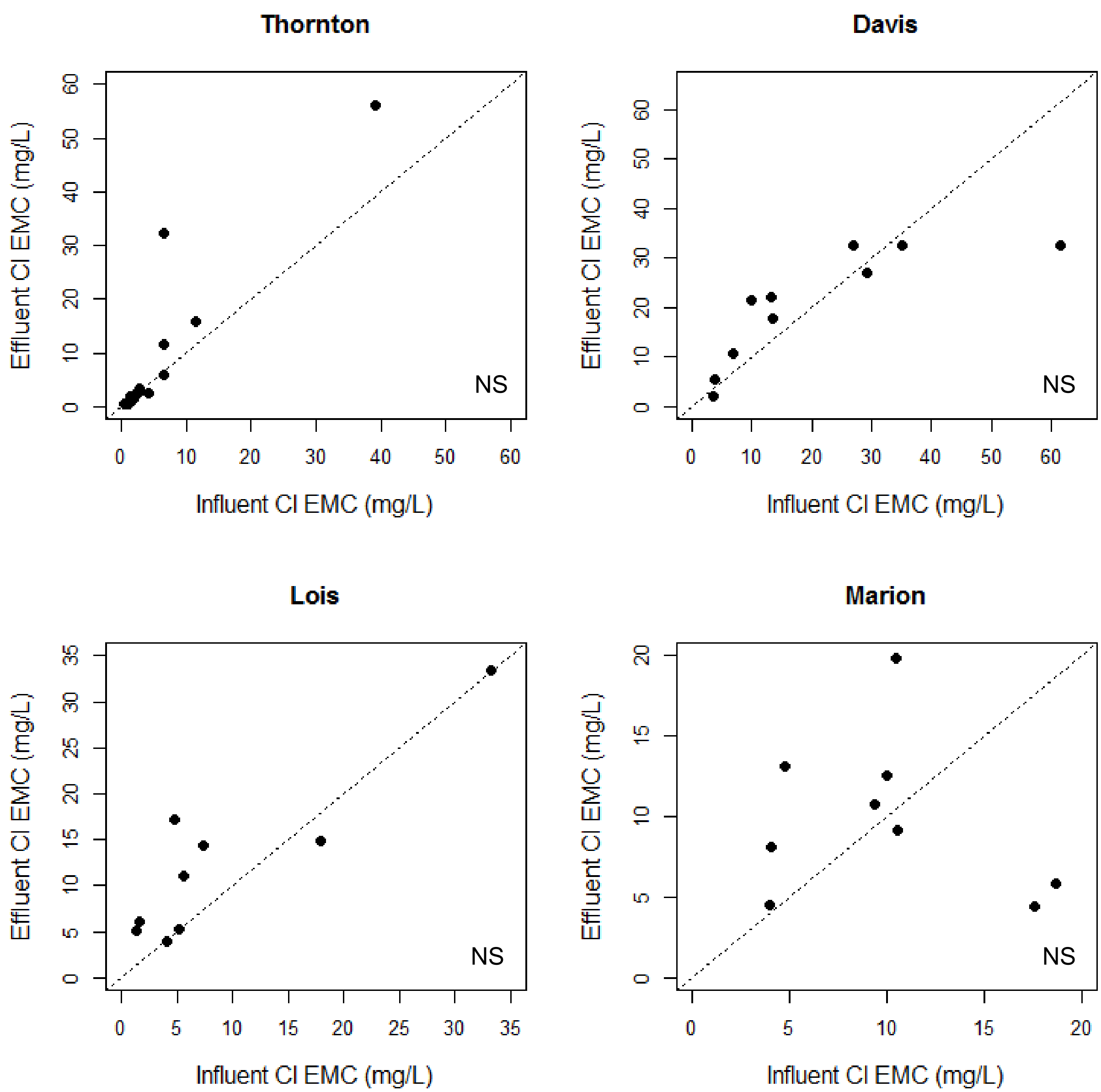

FIGURE 8. EFFLUENT CL EMC VS. INFLUENT CL EMC PLOTS FOR EACH WETLAND. DOTTED LINE REPRESENTS Y = X.

Site variables

Vegetation

Plant diversity in the constructed wetlands ranged from 0 to 24 species (Figure 9) with the highest diversity at Lois and the lowest at Davis. At Thornton and Marion the species were consistently distributed throughout the basin and only one plant community was identified (Figure 10 and Figure 13). The vegetation composition at Thornton was predominated by a mix of weeds and grasses, while Marion was mostly covered by cattail. At Lois, we identified five communities of plants throughout the basin. These communities were determined by identifying predominant species at different sections of the wetland. The five communities identified at Lois were: (1) ironwood and weeds, (2) grass, (3) moss and grass, (4) cattail and grass, and (5) ironwood and grass (Figure 12). A list of the species identified at each site and community can be found in Table 5. While this list displays information plant diversity, it does not reflect species abundance. In addition to plant diversity, percent cover of vegetation was also recorded. These values range from 0% at Davis, to 100% at Marion and Lois (Figure 9).

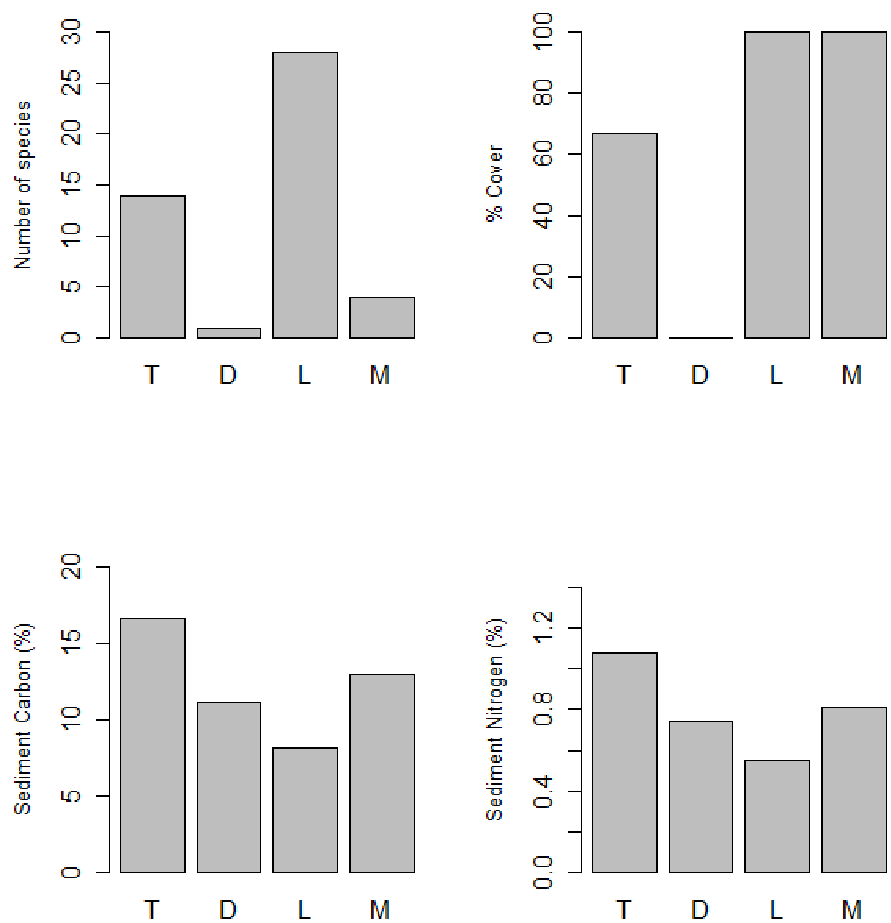

FIGURE 9. VEGETATION AND SEDIMENT CHEMISTRY DATA COLLECTED AT EACH SITE.

Legend

1 Grasses, weeds, and shrubs
2 Unvegetated pond

FIGURE 10. MAP SHOWING VEGETATION COMMUNITIES AT THORNTON.

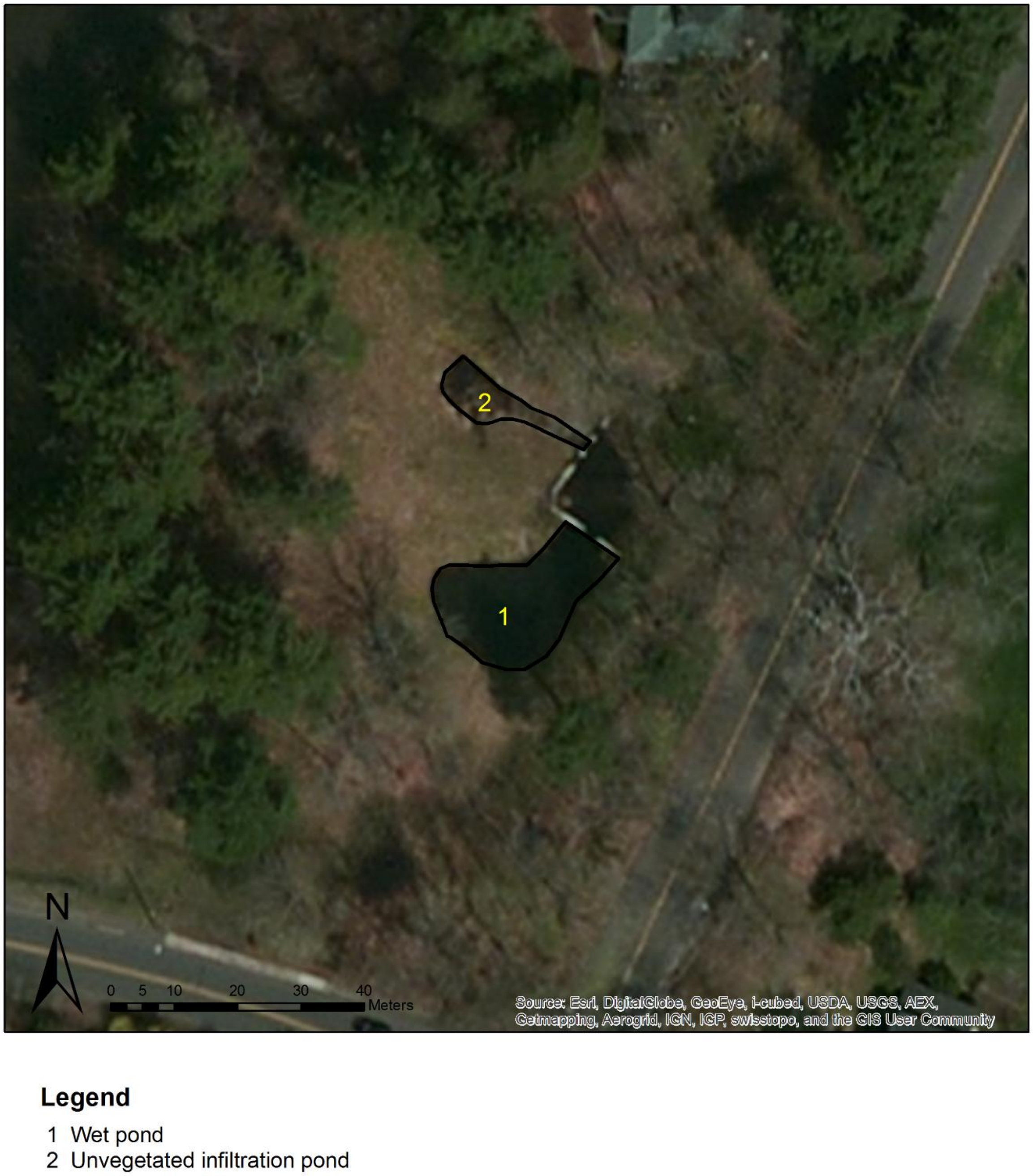

FIGURE 11. MAP SHOWING PONDS AT DAVIS.

FIGURE 12. MAP SHOWING VEGETATION COMMUNITIES AT LOIS.

Legend

1 Cattail

FIGURE 13. MAP SHOWING VEGETATION COMMUNITIES AT MARION.

TABLE 5. PLANT SPECIES BY COMMUNITY AT THORNTON, MARION, AND LOIS

Site	Community description	Species	Common name
Thornton	Weeds and grasses	*Alisma sp.*	Water Plantain
		Carex lurida	Shallow Sedge
		Carex sp.	Sedge
		Carpinus caroliniana	Ironwood
		Eleocharis sp.	Spikerush
		Impatiens capensis	Jewelweed
		Peltandra virginica	Arrow Arum
		Pontederia cordata	Pickerelweed
		Salix Nigra	Black Willow
		Typha sp.	Cattail
		Vitis sp.	Grapevine
			Unidentified Weed 1
			Unidentified Weed 2
Marion	Cattail	*Lythrum salicaria*	Purple Loosetrife
		Polygonum sp.	Smartweed
		Solidago sp	Goldenrod
		Species	Common Name
		Typha sp.	Cattail
Lois	Ironwood and weeds	*Artemisia vulgaris*	Mugworth
		Carex tribuloides	Blunt Broom Sedge
		Carpinus caroliniana	Ironwood
		Impatiens capensis	Jewelweed
		Leersia oryzoides	Rice Cutgrass
		Liriodendron	Tulip Tree
		Lythrum salicaria	Purple Loosestrife
		Panicum dichotomiflorum	Fall Panicum
		Parthenocissus quinquefolia	Virginia Creeper
		Polygonum sagittatum	Arrowleaf Earthumb
		Rubus sp	Blackberry
		Salix Nigra	Black Willow
		Typha Sp	Cattail
		Vitis sp.	Wild Grape
			Unidentified Weed 3
	Grass	*Carex sp.*	Sedge
		Carpinus caroliniana	Ironwood
		Juncus effusus	Soft Rush
		Leersia oryzoides	Rice Cutgrass
		Lythrum salicaria	Purple Loosestrife
		Onoclea sensibilis	Sensitive Fern

		Polygonum sagittatum	Arrowleaf Tearthumb
		Scirpus cyperinus	Woolgrass
		Solidago sp	Goldenrod
		Typha sp	Cattail
			Unidentified Weed 3
	Moss and gras	*Carpinus caroliniana*	Ironwood
		Juncus effusus	Soft Rush
		Lythrum salicaria	Purple Loosestrife
		Onoclea sensibilis	Sensitive Fern
		Polygonum sagittatum	Arrowleaf Tearthumb
	Cattail and grass	*Carex lurida*	Shallow Sedge
		Typha Sp	Cattail
		Panicum dichotomiflorum	Fall Panicum
		Polygonum hydropiperoides	Mild Water Pepper
		Lythrum salicaria	Purple Loosestrife
		Leersia oryzoides	Rice Cutgrass
		Rubus sp	Blackberry
		Polygonum sp.	Smartweed
		Liriodendron	Tulip Tree
			Unidentified Weed 3
	Ironwood and grass	*Carex sp.*	Sedge
		Carpinus caroliniana	Ironwood
		Impatiens capensis	Jewelweed
		Juncus effusus	Soft Rush
		Leersia oryzoides	Rice Cutgrass
		Liriodendron	Tulip Tree
		Lythrum salicaria	Purple Loosestrife
		Mimulus sp	Monkey Flower
		Onoclea sensibilis	Sensitive Fern
		Polygonum sagittatum	Arrowleaf Tearthumb
		Scirpus atrovirens	Green Bulrush
			Unidentified Weed 3

Sediment C and N content

Area-weighted average carbon concentration at the four sites ranged from 8.16 % to 16.89 %, with the highest value seen at Thornton and the lowest at Lois (Figure 9). At Thornton, we observed higher sediment carbon concentrations in the vegetated pond (Mean %C = 18.65) than in the unvegetated portion of the wetland (Mean %C = 12.98) (Table 6). Lois showed higher carbon concentrations in plant communities dominated by cattail and rice cutgrass (Mean %C = 10.64), while lower concentrations were observed in locations where ironwood was dominant (Mean %C = 3.95). At Marion, there is only one plant community, dominated by cattail. However, while the plant community is homogenous, carbon concentration across the wetland varied widely, ranging of 8.27 % to 19.83 %. Finally, at Davis, relatively high carbon concentrations were measured in the sediment even though the wetland is not vegetated. From the 10 points sampled, 7 had a concentration that exceeded 10%. An outlier of 1.23% C influences the mean concentration (11.65%), which is lower than the concentration at Marion. However, median carbon concentration at Davis is 13.44 %. Average N concentrations in wetland sediments ranged from 0.55% to 1.09% between sites. There is a high correlation (>0.90) between carbon concentration and N concentrations at all sites, resulting in similar trends to those observed for carbon.

Additional Variables

Wetlands were also characterized by design characteristics and influent N concentrations (Figure 14). Treatment ratios ranged from 0.22 to 2.17, with the lowest value at Thornton and the highest at Marion. The number of basins was the highest both at Thornton and Davis, with two main ponds at each site. In terms of influent N concentrations, no particular trend was observed. Thornton had the highest mean influent TN concentration, while Lois had the highest concentration for nitrate.

TABLE 6. SEDIMENT C AND N CONCENTRATIONS BY COMMUNITY AT EACH SITE.

Site	Community Description	Area (m^2)	Sediment Sample C (%)	Sediment Sample N (%)
Thornton	Grass and weeds	160	19.2	1.2
			20.6	1.4
			16.5	1.1
			20.1	1.4
			17.0	1.1
	Unvegetated pond	78	13.1	0.8
			6.8	0.4
			11.9	0.7
			17.7	1.0
			15.4	0.9
Davis	Wet Pond	294	13.6	0.9
			14.5	0.9
			1.2	0.1
			9.5	0.6
			14.9	0.9
	Unvegetated pond	71	16.7	1.2
			13.7	1.0
			13.3	0.9
			10.8	0.8
			8.3	0.6
Lois	Ironwood and weeds	124	7.6	0.5
			9.3	0.6
	Grass	329	4.9	0.3
			10.7	0.7
	Moss and grass	138	7.3	0.5
			7.4	0.5
	Cattail and grass	417	13.3	0.9
			8.0	0.6
	Ironwood and grass	93	3.8	0.3
			4.1	0.3
Marion	Cattail	456	19.8	1.2
			8.3	0.6
			7.0	0.4
			12.8	0.8
			12.8	0.8
			12.8	0.8
			14.2	0.8
			10.9	0.8
			18.2	1.0
			12.7	0.8

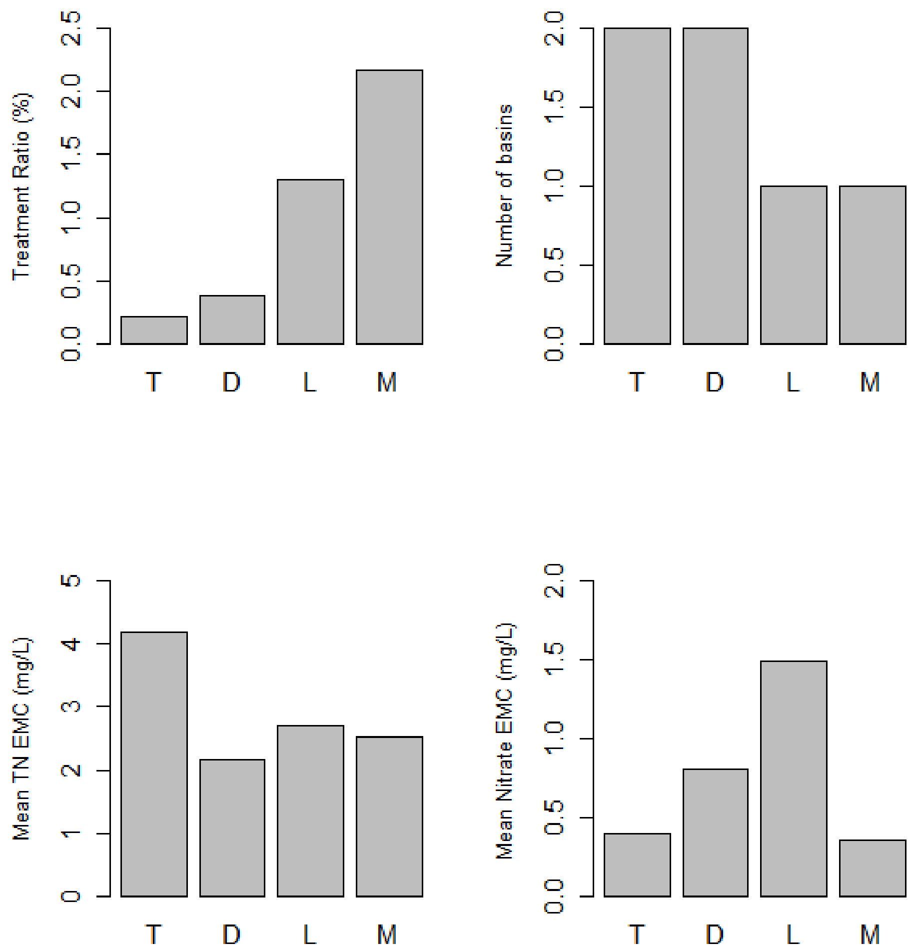

FIGURE 14. BAR PLOTS SHOWING DESIGN CHARACTERISTICS OF THE WETLANDS AND MEAN INFLUENT N CONCENTRATIONS.

STORM VARIABLES

Linear models were created using data from Davis and Thornton, the only sites that showed statistically significant N reduction. These models included influent water volume, maximum flow, influent N concentration, and mean water temperature as variables to predict N concentration change. All models suggest that mean influent N concentration is the only variable influencing N reduction ($p<0.001$) (Table 7 and Table 8). Mean water temperature, influent water volume, and maximum flow did not have a statistically significant influence on N concentration reduction. All models satisfied assumptions of normality and homoscedasticity. In general, the models show that higher N removal is observed when influent concentrations are higher (Figure 15A and Figure 15B).

TABLE 7. MULTIPLE LINEAR MODEL RESULTS FOR TOTAL NITROGEN REDUCTION

	Thornton			**Davis**		
Variable	*Estimate*	*Std. Error*	*p-value*	*Estimate*	*Std. Error*	*p-value*
Influent Water Volume	0.002	0.002	0.341	0.018	0.011	0.144
Mean Water Temperature	-0.028	0.035	0.432	0.024	0.063	0.717
Max Flow	-0.008	0.008	0.356	-0.028	0.026	0.310
Influent TN EMC	0.495	0.070	**0.000**	1.012	0.079	**0.000**
			R^2= 0.81			**R^2= 0.97**

TABLE 8. MULTIPLE LINEAR MODEL RESULTS FOR NITRATE REDUCTION

	Thornton		
Variable	*Estimate*	*Std. Error*	*p-value*
Influent Water Volume	0.000	0.000	0.653
Mean Water Temperature	-0.004	0.008	0.635
Max Flow	-0.003	0.002	0.105
Influent NO3 EMC	0.513	0.108	**0.000**
			R^2= 0.71

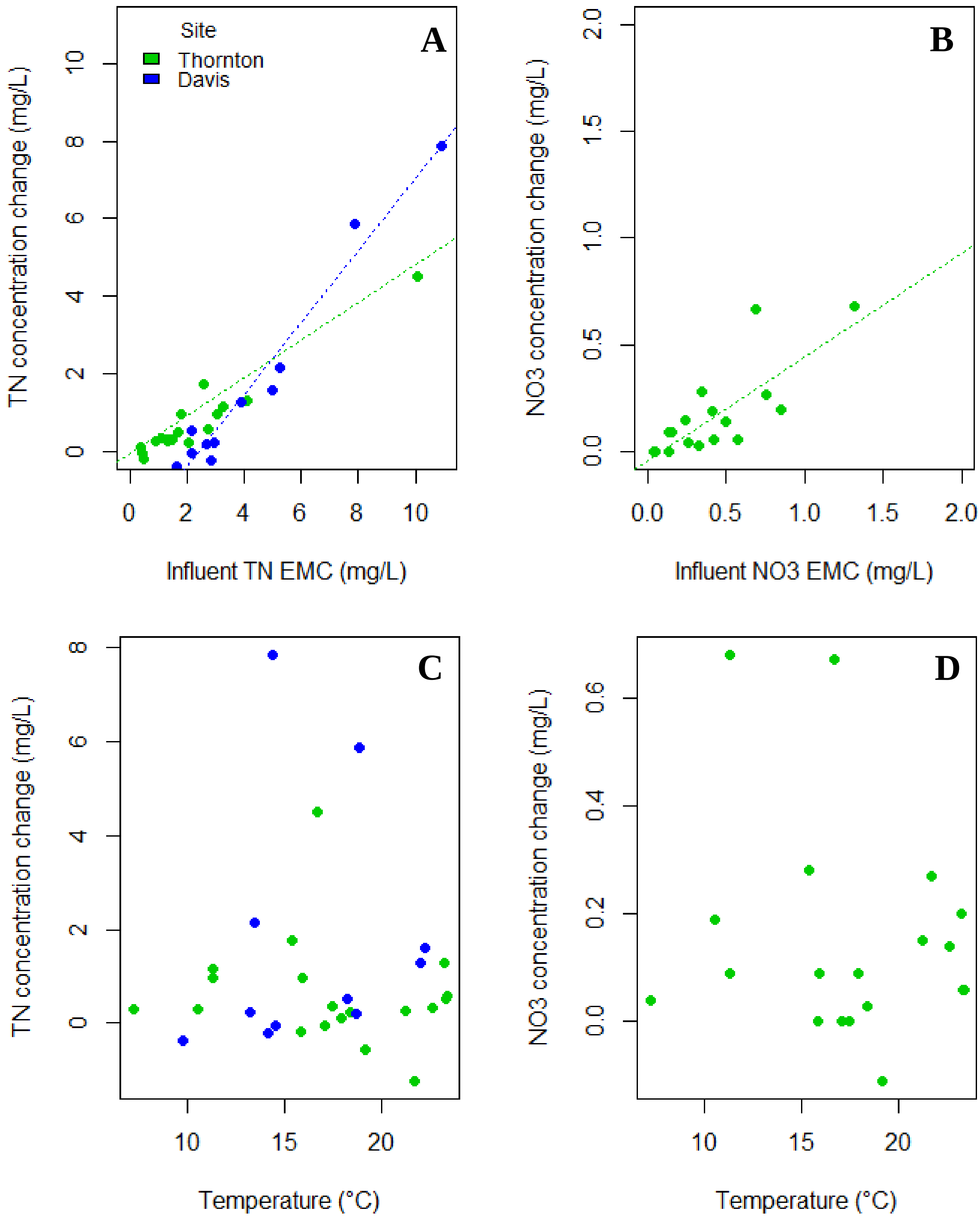

FIGURE 15. A. INFLUENT TN EMC VS. TN EMC REMOVAL AT DAVIS AND THORNTON. **B**. INFLUENT NITRATE EMC VS. NITRATE EMC REMOVAL AT THORNTON. **C**. TN EMC REDUCTION AT THORNTON AND DAVIS PLOTTED AGAINST TEMPERATURE. **D**. NITRATE EMC REDUCTION AT THORNTON PLOTTED AGAINST TEMPERATURE**.**

DISCUSSION

This study emphasizes the influence of design and placement of constructed wetlands in their N reduction performance. In general, our sites displayed variations in vegetation, sediment chemistry, design, and influent stormwater characteristics, resulting in variable N reduction effectiveness. From the four sites studied, two sites, Thornton and Davis, showed statistically significant concentration reduction of TN, while only Thornton displayed significant reduction of NO_3^-. Even though our study did not find significant reduction of NO_3^- at Davis, a previous study (unpublished) determined that nitrate was removed effectively from this site. This discrepancy is possibly a result of the small storm number assessed in our study and the lack of relatively large storms during our study period.

The only noticeable variables at Thornton and Davis that could be contributing to their N concentration reduction effectiveness are: (1) the number of treatment basins and (2) sediment carbon concentrations. Both Thornton and Davis contain two treatment basins that serve different purposes. At Davis, water travels through a large open infiltration pond that increases residence time, and later moves to a smaller infiltration area that was originally vegetated. At Thornton, water travels through a small unvegetated pond before reaching the vegetated area of the wetland. These results are consistent with previous studies that have shown that wetland heterogeneity and interspersion between open water and vegetation increase pollutant removal (Thullen et al. 2002, Ibekwe et al. 2007). Wetland heterogeneity promotes algal uptake by allowing sunlight penetration in open water ponds, increases the diversity of microbial populations, and allows mixing and aeration of floodwater. At Davis and Thornton, where influent NO_3^- concentrations are a small fraction (~20%) of the total influent N, aeration of the water column is important to promote N transformation processes (e.g. ammonification, nitrification) that will ultimately lead to N removal through denitrification.

In addition to the number of treatment basins, sediment carbon concentrations were generally high at both sites. Even though sediment carbon at Davis was lower than Marion's, an outlier from a sample collected at the edge of the wetland influenced the mean carbon concentration. In addition, comparing these sites with Marion is not ideal, since we suspect a design flaw is the main reason for Marion's ineffectiveness. The inlet and the outlet are approximately 7 m apart, resulting in residence times of a few minutes, thus preventing any biogeochemical transformation of N. Nevertheless, at Thornton and Davis, it is possible that high carbon concentrations are promoting greater denitrification rates by providing a source of energy to denitrifying bacteria, therefore promoting greater N reduction. Lois, which did not show statistically significant N removal, had relatively low soil carbon concentrations suggesting that denitrification could be carbon limited. Carbon limitation has been observed in sites with particularly high nitrate loads (Gale et al. 1993, Bachand and Horne 2000) and, at Lois, nitrate concentrations were relatively high compared to the rest of the sites.

Contrary to many studies in which vegetation has been found to significantly influence N removal (Bachand and Horne 2000, Picard et al. 2005, Ruiz-Rueda et al. 2009), vegetation does not seem to be having an effect on overall N reduction at these sites. Davis, although vegetated by cattail in the past, is currently not vegetated and is similar to a detention basin. In addition, Lois, which had both high vegetation cover and diversity, did not show statistically significant reduction of N. It is possible that N uptake by plants is not a dominant mechanism for N reduction at these sites. This has been suggested in previous studies in which denitrification has been found to influence N removal more than plant uptake (Bachand and Horne 2000, Vymazal 2007).

Most storm variable factors also seem to have no influence in N reduction. Based on the multiple linear model, maximum and total flow (as a proxy for storm size and intensity) were not significant predictors of N reduction. Surprisingly, water temperature, which has been found to influence N removal by many studies (Bachand and Horne 2000, Kadlec and Reddy 2001, Braskerud 2002, Picard et al. 2005), did not influence the reduction of N concentrations at our study sites. This suggests that during spring and summer months, when N removal should be the highest, another factor is limiting denitrification rates or, alternatively, that offsetting processes are confounding the temperature effects and maintaining constant N reduction throughout the year.

The only storm variable that we found to be influencing N reduction is influent N concentration. At both Thornton and Davis, higher influent mean concentrations resulted in higher N reduction. This finding has implications for constructed wetland site selection and highlights the importance of understanding the composition of stormwater before the wetland design phase. Although placing a constructed wetland at a location with relatively high stormwater N concentrations does not guarantee its effectiveness, if its design is favorable for biogeochemical N removal, higher performance could potentially be achieved.

CONCLUSIONS

This study contributes to the knowledge of constructed wetlands as a stormwater management practice and provides information on the underlying factors that promote biogeochemical N removal. In particular, our research highlights the relevance of constructed wetland design for achieving optimal N concentration reductions in stormwater. Based on our results we recommend designing interspersed wetlands that offer more opportunities for a variety of biogeochemical processes to occur and using sediments with high carbon concentrations to promote denitrification.

These findings are of particular relevance to the state of CT, where this practice is commonly implemented to reduce N loads without any information on their performance in the region. The information presented in this study contributes to the limited knowledge of constructed wetland design in this area and, if these factors are considered when designing constructed wetlands, can contribute to reduced nitrogen loads to the Long Island Sound.

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